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RESEARCH ARTICLE

Antidiabetic Activity of *Vasant Kusumakar Ras* in Streptozotocin and High Fat Diet Induced Type 2 Diabetes Mellitus in Sprague Dawley Rats

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Abstract

Background: *VasantKusumakar Ras (VK Ras)* is a traditional Ayurvedic preparation used in the treatment of Type-2 diabetes mellitus. Despite its clinical anti-diabetic claims, no pre-clinical attempts were made to rule out its efficacy as an antidiabetic agent. **Objectives:** The present study was carried out to find the anti-diabetic effect of *VK Ras* against a High-Fat Diet (HFD), and low-dose streptozotocin (STZ) induced type 2 diabetes and to explore the mode of action of *VK Ras*. **Materials and Methods:** Different doses of *VK Ras* were administered to diabetic rats for 35 days. The biochemical markers analysis, intestinal glucose uptake, and liver glycogen content were estimated at the end of the study and also vital organs were weighed and subjected to histopathological evaluation. **Results:** *VK Ras* treatment reduced blood glucose in a dose-dependent manner. The insulin, HbA1C, HOMA-IR, and lipid profiles were improved in *VK Ras-treated* animals as compared to diabetic control animals. The relative organ weights were changed in diabetic rats, and treatment with *VK Ras* corrected the organ weights. Intestinal glucose uptake and liver glycogen content were decreased with treatment. Further, the histopathological analysis of the pancreas and other vital organs had shown that dose-dependent restoration of organ function with *VK Ras* treatment. **Conclusions:** *VK Ras* treatment reduces insulin resistance as well as corrects the lipid, hepatic and renal abnormalities that arise from diabetes, these effects may be mediated by interfering with glucose transport from the gut and insulin release from the β pancreatic cells.

Keywords: HbA1C, HFD, HOMA-IR, Insulin, STZ, *VK Ras*

Abbreviations:

ANOVA: Analysis of Variance, AST: Aspartate Amino Transferase, ALT: Alanine Amino Transferase, ALP = Alkaline Phosphatase, Bd. wt: Body Weight, CHO: Cholesterol, CREA: Creatinine, ELISA: Enzyme Linked Immuno Sorbent Assay, F: Female, Fig.: Figure, GLU: Glucose, HDL: High Density Lipoprotein, HbA1c: Glycosylated Haemoglobin, HFD: High Fat Diet, HOMA-IR: Homeostatic Model of Assessment-Insulin Resistance, IAEC: Institutional Animal Ethics Committee, LDL: Low Density Lipoprotein, M: Male, NIN: National Institute of Nutrition, NPD: Normal Pellet Diet, OGTT: Oral Glucose Tolerance Test, P.O: Per Oral, RH: Relative Humidity, SD: Sprague Dawley, SEM: Standard Error of Mean, STZ: Streptozotocin, TG/TRI: Triglycerides, VEGF: Vascular Endothelial Growth Factor, VK Ras: *Vasant Kusumkar Ras*, VLDL: Very Low-Density Lipoprotein

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from either defective insulin secretion or action. Generally, diabetes mellitus is symptomized by polyphagia, polydipsia, polyuria, and blurred vision. Uncontrolled diabetes mellitus could lead to diabetic coma. Increased lipolysis in response to insulin deficiency, causes elevation of circulating ketone bodies. This in turn results in acidosis or ketoacidosis or nonketotic

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hyperosmolar syndrome[1]. Diabetic ketoacidosis is the triggering factor for multiorgan failure and mortality in diabetic subjects. Retinopathy, nephropathy, peripheral neuropathy, foot ulcers, amputations, and Charcot's joints are the long-term complications associated with diabetes. Studies have shown that diabetic subjects have a higher risk of vascular complications such as atherosclerosis, peripheral arterial disease and cerebrovascular disease[2].

The traditional Indian medicine system Ayurveda roots trace back to 2000 BC. The patronage towards ayurvedic therapies is snowballing due to their effectiveness as well as safety. World Health Organization began to understand the benefits of traditional medicines in the late 1970s and initiated to promote the concepts of Ayurveda[3]. The mentioning of Diabetes mellitus or Madhumeha as it is known to the ancient Indian physicians can be found in classical texts such as *Charakasamhita, Sushrutasamhita, Ashtanga sangraha, Madhava idana* and *Yoga ratnakara*. The most notable ayurvedic preparations for the treatment of diabetes mellitus or madhumeha are '*Vasanta Kusumakar Ras*' and '*Chandraprabhavati*'. Ayurvedic formulations for the treatment of diabetes are mostly composed of amalaki, triphala, fenugreek turmeric, neem, coccinea indica, bitter gourd, rose apple and cinnamon etc[4,5].

Vasant Kusumakar Ras is a herbo-mineral formulation known for anti-diabetic activity. It contains, *Svarna, Rajata, Vanga, Naga, Kantaloha, Abhraka, Pravala* and *MouthikaBhasmas* along with herbal components such as *Saccharum officinarum, Adhatodavasica, Santalum album, Vetiveriazizaniodes, Pavonia odorata, curcuma longa, Musa paradisiaca, Nelubiumspeciosum* and *Jasminum officinale*. As per the Ayurvedic texts, *Vasant Kusumakar Ras*possess anti-diabetic, immune protective properties[6]. Studies have shown that diabetic retinopathy in rats was prevented by *Vasant Kusumakar Ras* by inhibiting VEGF expression[7]. These findings have further affirmed the significance of *Vasant Kusumakar Ras* for the management of diabetic complications. Although this study has mentioned that at a dose of 11.5mg/kg, *Vasant Kusumakar Ras could* not reduce blood glucose as well as HbA1C levels. Animal models for type 2 diabetes are supposed to reflect insulin resistance with decreased insulin secretion rather than total beta cell damage as observed in the type 1 phenotype. Although Non-Obese type 2 diabetes models such as Goto–Kakizaki (GK) rats and hIAPP mice are employed in diabetes research, they are limited in usage due to their inability to mimic the human pathological scenario. Besides genetically modified models such as Lepob/ob or Leprdb/db, TallyHo/Jng and KK mice or ZDF, OLETEF and NoncNZO10/LtJ rats, Diet manipulated models such as high-fat diet-fed rats and mice gained popularity for their low-cost, reproducible and presession[8]. However, only High Fat Diet feeding in mice exhibited increased enhanced insulin secretion, pancreatic β-cell mass, and exocytosis[9]. Henceforth, administration of low-dose streptozotocin (35mg/kg) after priming the pancreatic beta cells with a significant duration of high-fat diet administration cannot produce extensive destruction of the beta cell, rather produces mild to moderate hypoinsulinemia and hyperglycemia. This stands as an explanatory mechanism behind the successful induction of type 2 diabetes with a High-Fat Diet low dose streptozotocin model.

Concerning the study reported by Tamoli SM *et al*., it is observed that the authors have employed a single dose streptozotocin (45mg/kg) induced diabetes induction model. A single dose of streptozotocin at a dose ranging from 40mg/kg to 70mg/kg without pancreatic beta cell priming with diet modification, could result in extensive beta cell damage and induces type 1 diabetes[10]. This could be the reason behind the non-reduction in blood glucose as well as HbA1C levels as observed in the study. Nonetheless, the study has highlighted the cytoprotective role of *Vasant Kusumakar Ras* in in the prevention of diabetic retinopathy. Henceforth, the current study has chosen a high-fat diet and streptozotocin model over a single dose of streptozotocin. The duration of high-fat diet treatment and dose of streptozotocin were considered from the previously published reports[11].

2. Materials and Methods

2.1 Procurement of Drug

Vasant Kusumakar Ras was procured from M/S. Dhootapapeshwar Ltd, Mumbai, India (Batch No. P200900133; MFG. LIC. Number: AYU-150; Date of Manufacture: 09/2020 and Date of Expiry: 08/2025). The drugs were kept under room temperature and humidity of 52 ± 10 % RH until the experiment gets completed.

2.2 Properties of the Drug

Physicochemical properties, Heavy metal analysis and Microbial analysis reports were supplied by the manufacturer.

2.2.1 Supplementary Data

Colour: Light brown to dark brown
Loss on drying at 105°C: Not more than 5% Wt/Wt
Friability: Not more than 1% Wt/Wt
Disintegration time: Not more than 30 min
Hardness: Not less than 1.5 Kg/cm^2
Average weight: 156 mg±7.5
pH: 6.8-7.2

Solubility: The solubility of the *Vasant Kusumakar Ras* powder was analyzed with various solvents such as cold, warm deionized water, alcohol, hydro alcohol (50:50) and honey. It showed maximum solubility with the distilled water alone.

Gold (Au)	: 3.2-4.5 mg per tablet
Lead (Pb)	: 3.5-5.5 mg per tablet
Silver (Ag)	: 2-5 mg per tablet
Iron (Fe)	: 3.5-5.5 mg per tablet
Mercury (Hg)	: 5-8.5 mg per tablet
Calcium (Ca)	: Not less than 9 mg per tablet
E. coli	: Absent
P. aeuroginosa	: Absent
Salmonella Sp.	: Absent
Staphylococcus Sp	: Absent
Total Microbial Plate Count	: Not more than 10^8 c.f.u per g
Total Yeast and Mould	: Not more than 10^8 c.f.u per g
Aflotoxcins (B1,B2,G1,G2)	: Complies as per API

2.3 Animals

This study was approved by Institutional Animal Ethics Committee (IAEC/NARIP/2018-19/02/Extn dated 13-01-2020), NARIP, Cheruthuruthy. Male and female SD rats were procured from a small animal veterinary breeding station, in Mannuthy, Thrissur, Kerala. Rats were housed individually in cages designed for rats.

2.4 Feed And High Fat Diet

HFD was procured from NIN, Hyderabad. The composition of High Fat Diet as described by Sreenivasan, *et al.*,[11].

2.5 Induction of Diabetes

Rats were maintained on High Fat Diet for 2 weeks. On day 15th, a low dose of streptozotocin (35 mg/kg) was injected intraperitoneally. Body weights, blood glucose, lipid, liver and kidney markers were checked post 1 week of streptozotocin administration (on 21 days), and another group of rats (vehicle/normal control) maintained with a normal pellet diet for the same duration without streptozotocin intervention.

2.6 Experimental Design and Treatment Schedule

After confirmation of diabetes, the animals were randomized based on their blood glucose levels. A total of 36 rats Male and Female were selected for the study and distributed into 06 groups, each group consisting of 6 animals (03 male + 03 female). The study design was mentioned as follows

NCM/NCF - Control
DCM/DCF - Disease Control
HVKM/HVKF - High dose *VK Ras* (25 mg/kg),
AVKM/AVKF - Average dose *VK Ras* (12 mg/kg),
LVKM/LVKF - Low dose *VK Ras* (6.5 mg/kg),
STDM/STDF - Standard drug (10 mg/kg Glibenclamide)

A dose volume (10 mL/kg bd.wt/p.o) of the vehicle was administered to all the animals in the control group from day 1 to day 35 (post streptozotocin administration). The test drug was prepared in distilled water and the dose was calculated based on the human dose (mg/kg) to rat conversion factor (6.2). Vehicle, test drug and standard drugs were administered daily for 35 days (05 weeks) from day 22 of the study i.e., 7 days post streptozotocin injection. Blood glucose levels, body weight, feed and water intake were measured.

During the last week of the trial Oral Glucose Tolerance Test (OGTT) was carried out by administering glucose (2 g/kg, P.O.) Blood glucose was analysed prior (0 h) and at

1, 2 and 3 hr after glucose loading with Accu check blood glucose monitoring strips by puncturing the tail vein.

2.7 Biochemical Estimation

A blood sample was collected at end of the study by puncturing the retro-orbital plexus and it was subjected to analysing of the serum lipid profile, and markers of liver and kidney function.

2.8 Glycosylated Haemoglobin (HbA1C)

HbA1C levels in the blood were measured by using ELISA kit (ELKBiotech, Ct.No ELK5370) as per the manufacturer's instructions.

2.9 Insulin

Plasma insulin levels were determined at the termination of the study using ELISA kit (BT LAB, Ct.No. E0707 Ra) as per the manufacturer's instructions.

2.10 HOMA IR

Insulin resistance (HOMA – IR) was calculated as per the equation

HOMA – IR= (CFNS × CFBG) / 22.5

Wherein CFNS is fasting serum insulin concentration (ng/ml) and CFBG is fasting blood glucose concentration (mmol /L).

2.11 Intestinal Glucose Uptake Assay

Intestinal glucose uptake was determined as per the method of Doluiso, *et al*. During the last day of the study, post euthanasia, 10 cm of the jejunum was removed, inverted, washed with cold Phosphate buffer saline and clamped at one end. The jejunum was filled with glucose-free DMEM and clamped at the other end. The jejunum was inverted and placed in DMEM containing 20 mmol/l glucose. Aliquots were taken from the DMEM within the intestine and glucose concentration in the samples was determined with a glucose oxidase method[12].

2.12 Liver Glycogen Content Estimation

Liver glycogen content will be determined using the method of Van der Vies. Filtrate from the liver tissue ground with 5% TCA was boiled along with KOH for an hour. The mixture was neutralized with glacial acetic acid. Slow addition of 2 ml of this mixture to a test tube containing 4 ml anthrone reagent kept in a boiling water bath results in the development of colour. The optical density of the same was read at 650 nm using a spectrophotometer against a blank containing TCA[13].

2.13 Histopathology

Animals were euthanized under CO2 asphyxia and a detailed post-mortem was carried out. The heart, liver, kidneys, soleus and extensor digitorus muscle were weighed. The heart liver, kidney and pancreas were preserved in 10% formalin for histopathology analysis.

2.14 Statistical Analysis

Results obtained from the present study were expressed as Mean ±SEM. Data were subjected to statistical analysis through one-way analysis of variance (ANOVA) with multiple comparisons using the dunnets test using Graph pad prism software. $p<0.05$ was considered statistically significant.

3. Results

3.1 Induction of Diabetes

After stabilizing the disease control group with 2 weeks of HFD the body weights were increased as compared to NPD-fed animals. The low dose of streptozotocin intervention reduced the body weight of HFD-fed rats and made the bd.wt similar to NPD-fed rats. Further significant increase in glucose ($p<0.0001$), Cholesterol ($p<0.01$), LDL ($p<0.0001$), TG ($p<0.0001$), and VLDL ($p<0.0001$) was noted along with a decrease in HDL ($p<0.0001$) as compared to the NPD fed rats. The increase in blood glucose and lipids confirms the onset of diabetic dyslipidaemia with the HFD+ low-dose streptozotocin model which mimics the similar physiological aspect of diabetes in humans. The liver enzyme and kidney marker levels also significantly (AST $p<0.05$, ALT $p<0.001$, ALP $p<0.0001$, Creatinine $p<0.001$) increased in HFD group rats as compared to the NPD rats which confirms the onset of hepatomegaly and renal complications with diabetes mellitus (Table 1 and Figure 1).

Table 1. Comparison of NPD and HFD-fed animals during the induction phase (before treatment)

Parameter	NPDM	HFDM	NPDF	HFDF
Bd. Wt (g)	295±04	297±03 ^{ns}	262±05	269±04 ^{ns}
Glucose(mg/dl)	77±03	353±07****	98±08	384±08****
HDL (mg/dl)	44±04	27±02**	57±04	20±02 ****
LDL (mg/dl)	26±02	190±11****	11±04	167±12****
VLDL (mg/dl)	15±02	62±06***	20±03	92±03****
Cholesterol (mg/dl)	80.0±2.0	234±28*	88±03	262±26**
Triglycerides(mg/dl)	77±08	308±29**	97±17	462±17****
Creatinine (U/L)	0.40±0.01	1.3±0.1***	0.37±0.01	1.33±0.12 **
AST U/L)	164±06	223±12 *	113±03	121±05 ^{ns}
ALT U/L)	56±04	146±09***	55±7.0	110±08**
ALP U/L)	279±78	1234±57****	267±43	758±65**

Values are expressed as mean ± SEM of n=3 NPD and n=15 for HFD, Parameters of HFD group were compared with the NPD group using 't' test, Level of significance expressed as *p<0.05; **p<0.01, ***p<0.001; ****p<0.0001; ^{ns}-Non significant

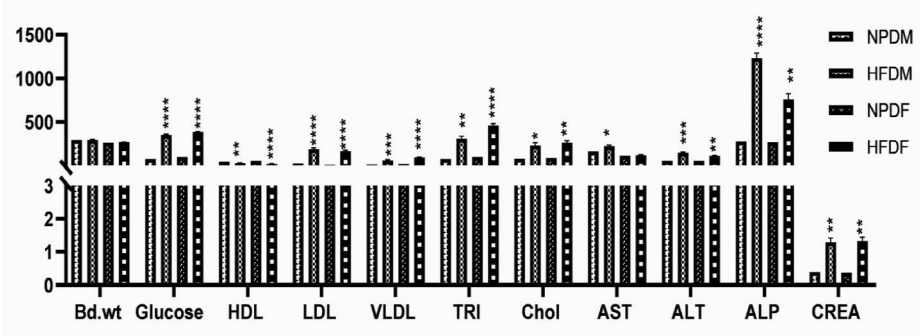

Figure 1. Comparison of NPD and HFD during the induction phase (before treatment).

4. Treatment

4.1 Feed Intake, Water Intake and Body Weight

The test drug-treated animals had shown a significant increase in feed intake (p<0.0001),, body weight (p<0.0001) and water intake (p<0.0001) as compared to the disease-control animals. (Table 2 and Figure 2).

4.2 Glucose and Lipid Levels

Up on treatment with test drug, a significant decrease in glucose (p<0.001), Cholesterol (p<0.01), triglycerides (p<0.001), LDL (p<0.01), VLDL (p<0.0001) levels and a significant increase in HDL levels were observed (p<0.05) (Table 3 and Figure 3).

4.3 Liver and Kidney Markers

Treatment with the test drug significantly reduced the serum AST levels (p<0.001), ALT (p<0.001), ALP (p<0.001) and creatinine (p<0.0001) levels as compared to the disease control (Table 4 and Figure 4).

4.4 Insulin, HOMA and HbA1c

High relative insulin resistance (HOMA) was observed in disease-control animals when compared to the normal control animals (p<0.001). The relative insulin resistance was significantly reduced (p<0.01)in test drug-treated animals as well as significant improvement HbA1C levels were observed (p<0.05). Insulin levels were improved with treatment however the results were not significant (p>0.05) (Table 5 and Figure 5).

Table 2. Body weight, feed and water intake after treatment

Group	Body weight (G)	Feed intake (G)	Water intake (mL)
NCM	388±09	148±03	220±11
DCM	260±04$^{@-****}$	88±05$^{@-****}$	367±16$^{@-****}$
HVKM	374±10$^{\$-****}$	134±04$^{\$-****}$	250±03$^{\$-****}$
AVKM	357±04$^{\$-****}$	128±03$^{\$-****}$	285±05$^{\$-**}$
LVKM	342±06$^{\$-****}$	120±01$^{\$-***}$	294±07$^{\$-**}$
STDM	346±05$^{\$-****}$	136±03$^{\$-****}$	256±18$^{\$-****}$
NCF	213±07	129±02	146±11
DCF	225±04$^{@-****}$	75±03$^{@-****}$	240±16$^{@-***}$
HVKF	287±07$^{\$-***}$	109±04$^{\$-***}$	165±11$^{\$-**}$
AVKF	300±04$^{\$-****}$	110±05$^{\$-***}$	180±06$^{\$-**}$
LVKF	285±04$^{\$-***}$	102±02$^{\$-**}$	222±10$^{\$-ns}$
STDF	301±06$^{\$-****}$	119±05$^{\$-****}$	157±09$^{\$-***}$

Values are expressed as mean ± SEM of 3 rats, Data was subjected to analysis through one-way ANOVA with dunnets multiple comparison test.**p<0.01; ***p<0.001; ****p<0.0001; ^{ns}-Non significant. @-comparsion of DCM with NCM and DCF with NCF $- comparison of test and standard drug-treated groups with DCM /DCF

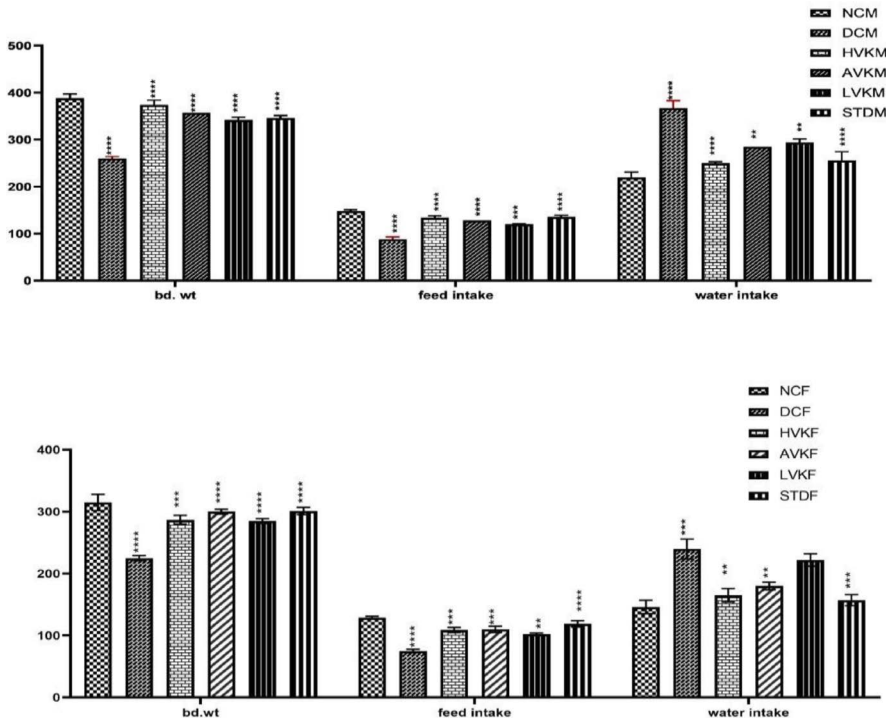

Figure 2. Body weight, Feed and water intake after treatment.

Table 3. Glucose and lipid levels after treatment

Group	Glu (mg/dl)	Chol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	TRI (mg/dl)
NCM	101±11	50±04	17±03	47±06	11±03	57±13
DCM	301±26$^{@-****}$	344±65$^{@-***}$	202±12$^{@-****}$	17±04$^{@*}$	73±06$^{@-****}$	362±28$^{@-****}$
HVKM	158±16$^{\$-***}$	172±15$^{\$-*}$	129±12$^{\$-**}$	45±04$^{\$-*}$	39±04$^{\$-****}$	196±19$^{\$-***}$
AVKM	180±14$^{\$-**}$	207±23$^{\$-*}$	146±16$^{\$-*}$	38±09$^{\$-ns}$	44±02$^{\$-****}$	221±09$^{\$-***}$
LVKM	191±18$^{\$-**}$	223±28$^{\$-ns}$	152±05$^{\$-*}$	33±01$^{\$-ns}$	51±04$^{\$-****}$	255±21$^{\$-**}$
STDM	105±15$^{\$-****}$	144±25$^{\$-**}$	108±09$^{\$-***}$	49±07$^{\$-**}$	26±02$^{\$-****}$	129±12$^{\$-****}$
NCF	84±08	73±03	10±01	52±03	11±01	56±03
DCF	339±28$^{@-****}$	338±35$^{@-****}$	221±13$^{@-****}$	23±06$^{@-*}$	88±05$^{@-****}$	438±24$^{@-****}$
HVKF	192±22$^{\$-**}$	197±15$^{\$-**}$	138±05$^{\$-**}$	47±06$^{\$-*}$	48±07$^{\$-**}$	242±35$^{\$-***}$
AVKF	196±35$^{\$-**}$	230±29$^{\$-*}$	182±23$^{\$-ns}$	44±06$^{\$-ns}$	54±08$^{\$-*}$	272±31$^{\$-**}$
LVKF	206±36$^{\$-*}$	243±27$^{\$-ns}$	195±15$^{\$-ns}$	39±08$^{\$-ns}$	64±03$^{\$-ns}$	318±14$^{\$-*}$
STDF	101±11$^{\$-***}$	149±15$^{\$-***}$	111±11$^{\$-***}$	51±08$^{\$-*}$	31±11$^{\$-***}$	155±32$^{\$-****}$

Values are expressed as mean ± SEM of 3 rats, Data was subjected to analysis through one-way ANOVA with dunnets multiple comparison test.**p<0.01; ***p<0.001; ****p<0.0001; ^{ns}-Non significant. @-comparsion of DCM with NCM and DCF with NCF $- comparison of test and standard drug-treated groups with DCM /DCF

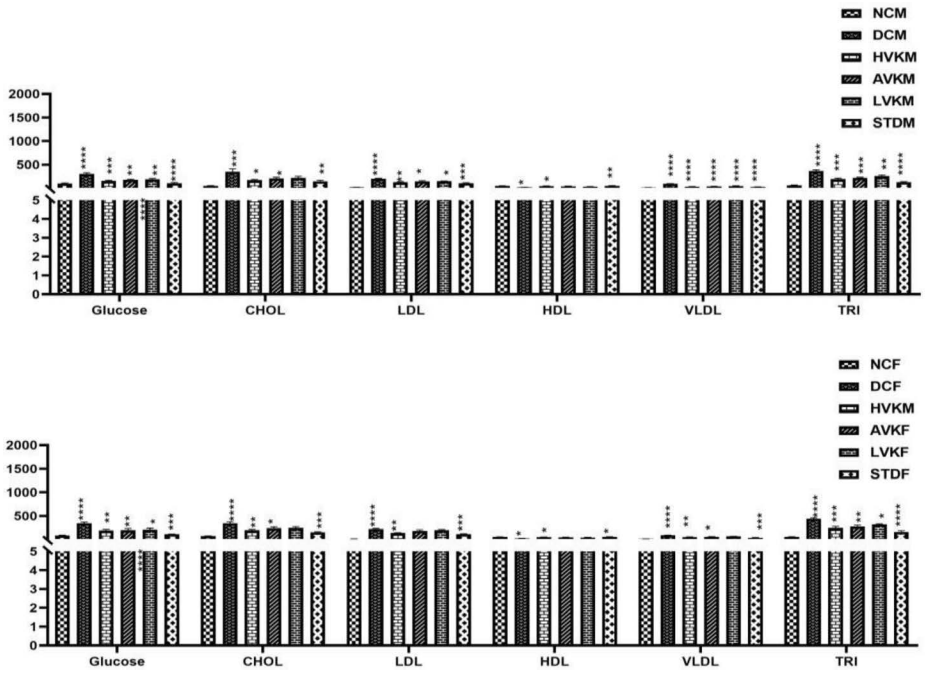

Figure 3. Glucose and lipid levels after treatment.

4.5 Intestinal Glucose Uptake and Liver Glycogen Content

The intestinal glucose uptake was significantly increased in disease-control animals when compared to the normal control animals (p<0.01), and treatment with a test drug significantly reduced intestinal glucose absorption (p<0.05). The liver glycogen content was significantly decreased in disease-control animals

Table 4. Liver and kidney markers after treatment

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	CREA (U/L)
NCM	121±01	69±15	301±79	0.36±0.01
DCM	354±43$^{@-***}$	264±36$^{@-****}$	1516±248$^{@-****}$	3.1±0.3$^{@-****}$
HVKM	165±23$^{\$-**}$	109±07$^{\$-***}$	591±41$^{\$-***}$	0.8±0.2$^{\$-****}$
AVKM	177±02$^{\$-**}$	123±02$^{\$-***}$	716±73$^{\$-**}$	1.4±0.1$^{\$-***}$
LVKM	183±33$^{\$-**}$	135±15$^{\$-**}$	748±128$^{\$-**}$	1.8±0.1$^{\$-**}$
STDM	135±26$^{\$-***}$	93±10$^{\$-****}$	446±39$^{\$-***}$	0.7±0.2$^{\$-****}$
NCF	90±13	90±10	348±63	0.34±0.02
DCF	269±38$^{@-****}$	251±49$^{@-**}$	1128±81$^{@-****}$	2.7±0.3$^{@-****}$
HVKF	115±10$^{\$-***}$	145±27$^{\$-*}$	541±37$^{\$-***}$	1.3±0.2$^{\$-**}$
AVKF	166±12$^{\$-n**}$	166±06$^{\$-ns}$	571±30$^{\$-***}$	1.3±0.1$^{\$-**}$
LVKF	169±08$^{\$-**}$	172±12$^{\$-ns}$	673±98$^{\$-**}$	1.3±0.4$^{\$-**}$
STDF	104±09$^{\$-***}$	104±07$^{\$-*}$	344±32$^{\$-****}$	0.9±0.1$^{\$-***}$

Values are expressed as mean ± SEM of 3 rats, Data was subjected to analysis through one-way ANOVA with dunnets multiple comparison test.**p<0.01; ***p<0.001; ****p<0.0001; ^{ns}-Non significant. @-comparsion of DCM with NCM and DCF with NCF
$- comparison of test and standard drug-treated groups with DCM /DCF

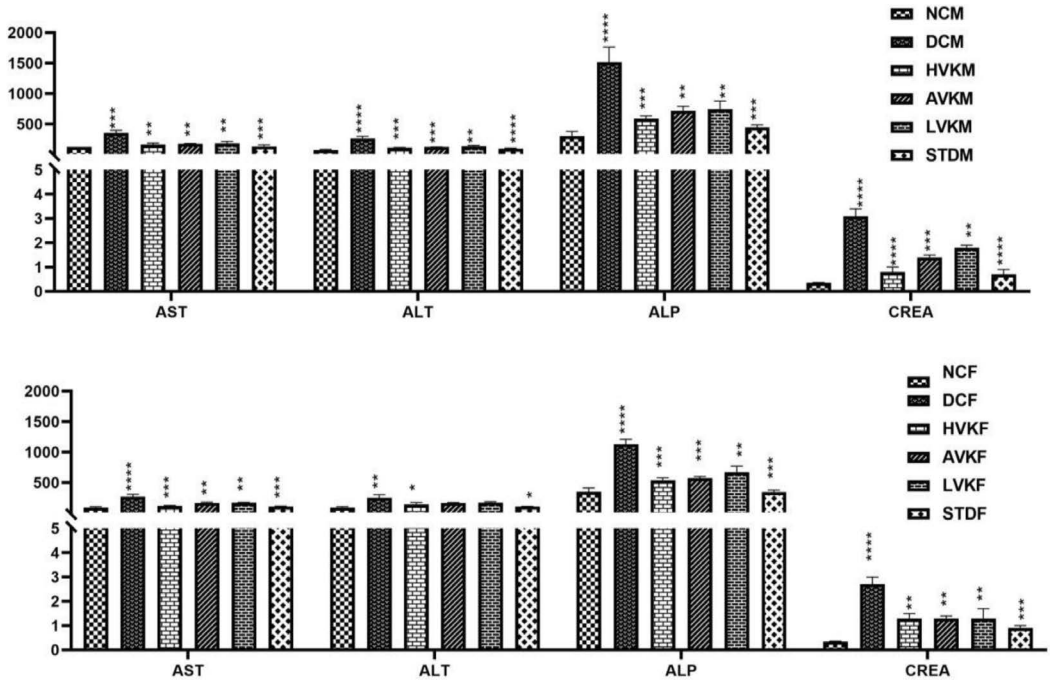

Figure 4. Liver and kidney markers after treatment.

Table 5. Insulin, HOMA and HbA1c after treatment

Group	Insulin (IU/L)	HOMA	HbA1c (%)
NCM	6.6±0.4	1.6±0.1	1.9±0.3
DCM	3.5±0.2$^{@-*}$	4.0±0.5$^{@-***}$	5.8±0.6$^{@-***}$
HVKM	6.0±1.2$^{\$-ns}$	2.5±0.2$^{\$-*}$	3.0±0.3$^{\$-**}$
AVKM	5.0±0.9$^{\$-ns}$	2.2±0.5$^{\$-**}$	3.5±0.4$^{\$-*}$
LVKM	4.3±0.4$^{\$-n}$s	2.1±0.2$^{\$-**}$	3.8±0.5$^{\$-*}$
STDM	6.1±0.4$^{\$-ns}$	1.8±0.1$^{\$-**}$	2.4±0.4$^{\$-***}$
NCF	6.1±0.6	1.3±0.2	2.3±0.5
DCF	3.2±0.2$^{@-*}$	4.0±0.5$^{@-**}$	6.2±0.5$^{@-***}$
HVKF	5.3±1.0$^{\$-ns}$	2.5±0.5$^{\$-ns}$	3.5±0.4$^{\$-**}$
AVKF	4.6±0.8$^{\$-ns}$	2.3±0.8$^{\$-ns}$	3.1±0.5$^{\$-**}$
LVKF	4.7±0.5$^{\$-ns}$	2.4±0.3$^{\$-ns}$	3.7±0.5$^{\$-**}$
STDF	5.9±0.5$^{\$-*}$	1.6±0.1$^{\$-*}$	2.2±0.2$^{\$-***}$

Values are expressed as mean ± SEM of 3 rats, Data was subjected to analysis through one-way ANOVA with dunnets multiple comparison test.**p<0.01; ***p<0.001; ****p<0.0001; ^{ns}-Non significant. @-comparsion of DCM with NCM and DCF with NCF $- comparison of test and standard drug-treated groups with DCM /DCF

Table 6. Intestinal glucose uptake and Liver glycogen content after treatment

Group	Intestinal glucose uptake (mmol/l)	Liver glycogen content mg /2g of tissue)
NCM	3.16±0.33	100±15.5
DCM	8.27±0.52$^{@-**}$	41.0±11.2$^{@-*}$
HVKM	5.11±1.11$^{\$-*}$	65.5±12.8$^{\$-ns}$
AVKM	5.72±1.16$^{\$-ns}$	50.6±11.1$^{\$-ns}$
LVKM	6.81±0.58$^{\$-ns}$	49.5±12.5$^{\$-ns}$
STDM	4.26±0.15$^{\$-**}$	112±18.2$^{\$-*}$
NCF	3.56±0.41	94±11.2
DCF	9.10±0.20$^{@-**}$	47±8.6$^{@-*}$
HVKF	4.71±0.99$^{\$-*}$	61±14$^{\$-ns}$
AVKF	5.95±1.54$^{\$-ns}$	55±9.2$^{\$-ns}$
LVKF	6.56±1.11$^{\$-ns}$	44±10.1$^{\$-ns}$
STDF	4.15±0.15$^{\$-**}$	95±7.4$^{\$-*}$

Values are expressed as mean ± SEM of 3 rats, Data was subjected to analysis through one-way ANOVA with dunnets multiple comparison test.**p<0.01; ***p<0.001; ****p<0.0001; ^{ns}-Non significant. @-comparsion of DCM with NCM and DCF with NCF $- comparison of test and standard drug-treated groups with DCM /DCF

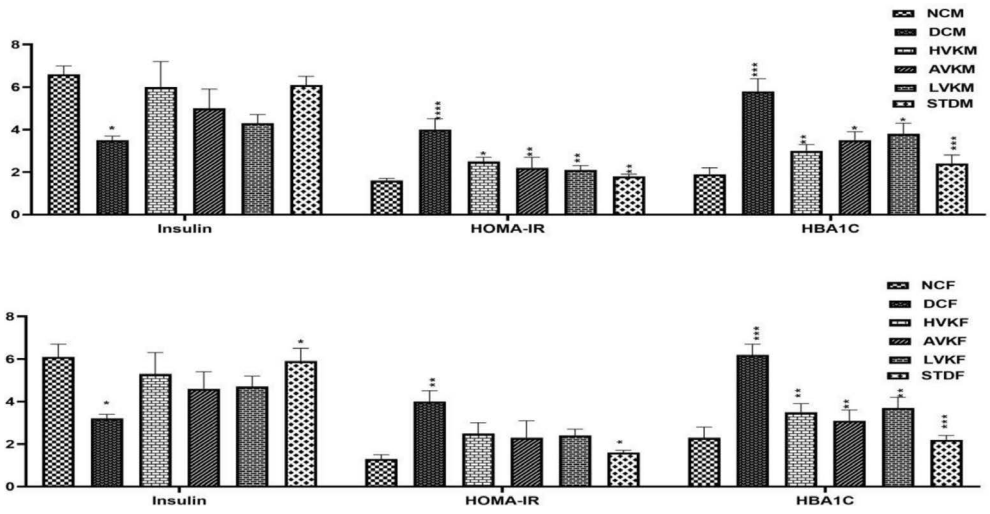

Figure 5. Insulin, HOMA and HbA1c after treatment.

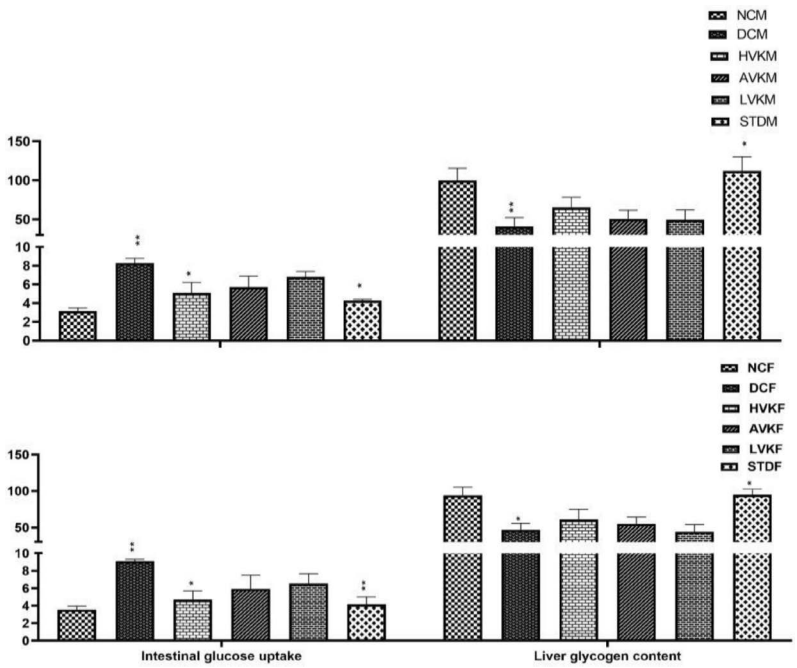

Figure 6. Intestinal glucose uptake and Liver glycogen content after treatment.

Table 7. Relative organ weight after treatment

Group	Liver	Heart	Kidney	Soleus muscle	Digitorus extensor longus muscle
NCM	2.2±0.07	0.3±0.01	0.5±0.04	0.090±0.006	0.20±0.01
DCM	5.4±0.3$^{@-****}$	0.55±0.03$^{@-ns}$	0.98±0.01$^{@-**}$	0.150±0.006$^{@-ns}$	0.11±0.010$^{@-ns}$
HVKM	2.8±0.06$^{\$-****}$	0.28±0.02$^{\$-ns}$	0.54±0.01$^{\$-**}$	0.070±0.002$^{\$-ns}$	0.153±0.015$^{\$-ns}$
AVKM	3.4±0.04$^{\$-****}$	0.4±0.02$^{\$-ns}$	0.56±0.06$^{\$-**}$	0.060±0.002$^{\$-ns}$	0.141±0.012$^{\$-ns}$
LVKM	4.3±0.34$^{\$-****}$	0.42±0.00$^{\$-ns}$	0.59±0.05$^{\$-*}$	0.050±0.002$^{\$-ns}$	0.160±0.010$^{\$-ns}$
STDM	2.6±0.14$^{\$-****}$	0.31±0.00$^{\$-ns}$	0.55±0.02$^{\$-**}$	0.050±0.01$^{\$-ns}$	0.160±0.023$^{\$-ns}$
NCF	2.53±0.14	0.3±0.03	0.60±0.02	0.09±0.009	0.182±0.040
DCF	4.77±0.19$^{@-****}$	0.5±0.01$^{@-ns}$	1.10±0.10$^{@-****}$	0.04±0.003$^{@-ns}$	0.120±0.090$^{@-ns}$
HVKF	3.4±0.05$^{\$-***}$	0.31±0.02$^{\$-ns}$	0.69±0.10$^{\$-***}$	0.06±0.001$^{\$-ns}$	0.135±0.011$^{\$-ns}$
AVKF	3.74±0.14$^{\$-****}$	0.37±0.01$^{\$-ns}$	0.82±0.0$^{\$-*}$	0.06±0.002$^{\$-ns}$	0.110±0.010$^{\$-ns}$
LVKF	4.28±0.05$^{\$-****}$	0.49±0.10$^{\$-ns}$	1.00±0.01$^{\$-ns}$	0.06±0.002$^{\$-ns}$	0.125±0.014$^{\$-ns}$
STDF	3.05±0.14$^{\$-****}$	0.37±0.01$^{\$-ns}$	0.63±0.05$^{\$-****}$	0.09±0.010$^{\$-ns}$	0.210±0.040$^{\$-ns}$

Values are expressed as mean ± SEM of 3 rats, Data was subjected to analysis through one-way ANOVA with dunnets multiple comparison test.**p<0.01; ***p<0.001; ****p<0.0001; ^{ns}-Non significant. @-comparsion of DCM with NCM and DCF with NCF
$- comparison of test and standard drug-treated groups with DCM /DCF

(p<0.05) but the test drug treatment has no significant effect on liver glycogen content (p>0.05) (Table 6 and Figure 6).

4.6 Relative Organ Weight

At the end of treatment, the heart, liver, kidneys, soleus muscle and extensor digitorum longus muscle were weighed. No significant changes in the relative weight of the heart, soleus and extensor digitorum longus muscle compared to the disease-control animals (p>0.05).

The relative weight of the kidneys (p<0.001) and liver (p<0.001) were significantly increased in disease-control animals when compared to the normal control animals.

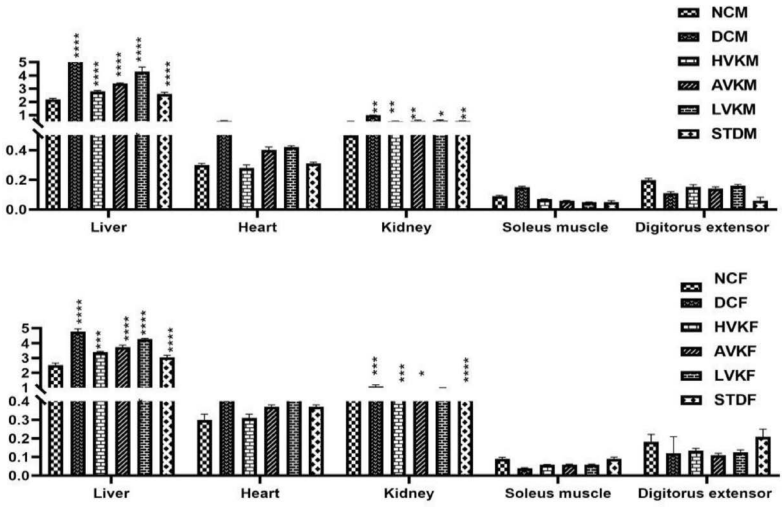

Figure 7. Relative organ weight after treatment.

Table 8. OGTT after treatment

Group	0 hr	1st hr	2nd hr	3rd hr
NCM	106±05	175±03^{a***}	140±10^{b-ns}	101±02^{c-ns}
DCM	363±29	446±22^{a****}	427±17^{b**}	408±18^{c*}
HVKM	161±08	238±05^{a***}	160±14^{b-ns}	158±04^{c-ns}
AVKM	172±14	232±04^{a**}	173±14^{b-ns}	158±13^{c-ns}
LVKM	180±08	239±03^{a**}	191±06^{b-ns}	166±09^{c-ns}
STDM	114±06	172±13^{a**}	112±02^{b-ns}	107±01^{c-ns}
NCF	96±03	176±12^{a****}	140±10^{b*}	99±01^{c-ns}
DCF	355±11	446±19^{a****}	426±18^{b****}	403±18^{c**}
HVKF	176±16	231±08^{a**}	171±11^{b-ns}	159±09^{c-ns}
AVKF	189±10	238±03^{a**}	174±03^{b-ns}	160±01^{c-ns}
LVKF	185±03	251±10^{a****}	198±05^{b-ns}	184±03^{c-ns}
STDF	118±05	180±04^{a***}	119±08^{b-ns}	104±01^{c-ns}

Values are expressed as mean ± SEM of 3 rats, Data was subjected to analysis through one-way ANOVA with dunnets multiple comparison test.**p<0.01; ***p<0.001; ****p<0.0001; ^{ns}-Non significant. a-comparison1st hr with 0th hr, b- comparison 2nd hr with 0th hr,b- comparison 3rd hr with 0th hr

The treatment with the test drug had shown significant improvement in the relative weight of the kidney (p<0.001) and liver (p<0.0001) (Table 7 and Figure 7).

4.7 Oral Glucose Tolerance Test (OGTT)

Normal control animals had exhibited raise in glucose after 1hr of glucose loading followed by a decline in glucose levels at 2nd and 3rd hr (normal homeostasis of glucose). The disease-control diabetic rats had shown increased levels of glucose in the blood till 3rd hr(Abnormal homeostasis of glucose). Test drug treatment had shown significant raise in the blood glucose levels at the 1st hr as compared to the 0th hr(p<0.0001), however, the rise in blood glucose levels was not significant at the 2nd and 3rd hr, when compared to 0th hr (Table 8 and Figure 8).

4.8 Histopathology

Histopathology evaluation of the pancreas, liver, kidney, and heart showed histopathological lesions which were predominantly reduced in number and size with degeneration of islets of Langerhans in the pancreas (Figure 9) and vacuolar changes in the tubular epithelium of the kidney (Figure 11), fatty change/ glycogen deposition associated vacuolar changes in the hepatocytes (Figure 10). There were no treatment-related histopathological changes in the heart (Figure 12).

In the present study, during the histopathology evaluation of the pancreas, injury to the islets of Langerhans was observed in the form of reduced number and size with degeneration/necrosis along with vacuolar changes in the liver and kidney. Pancreas and liver lesions from a high dose of *Vasant Kusumakar Ras* treated groups showed mild improvement in the form of regeneration/ change in size and number of Islets of Langerhans, and mild reduction in vacuolar changes in the liver and kidney when compared to the disease group.

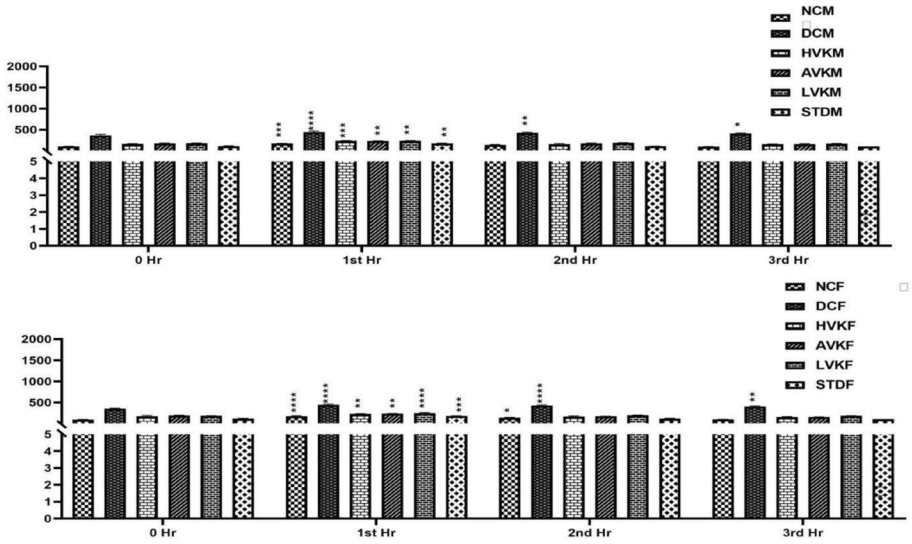

Figure 8. OGTT after treatment.

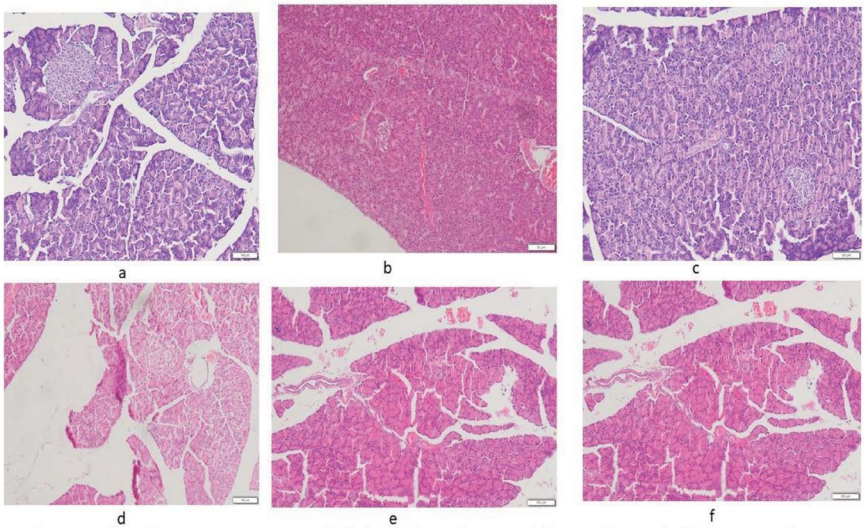

Figure 9. Effect of *VK Ras* treatment on pathological lesions of Pancreas **(a).** Normal control; **(b).** Streptozotocin (STZ + HFD) control; **(c).** High dose of *VK Ras*; **(d).** Average dose of *VK Ras*; **(e).** Low dose of *VK Ras;* **(f).** Glibenclamide.

5. Discussion

Body weights of the High Fat Diet fed animals increased from day 0 to day 14 further significant decline was observed post streptozotocin administration (Tables 1 and 2) and there was no significant weight loss observed in treatment groups (Table 2). Instead, treatment group animals showed a parallel growth with the normal pellet diet-fed fed group (Table 2). Although polyphagia is a notable symptom of type 2 diabetes, feed intake of the disease-control animals has significantly decreased after streptozotocin administration. This could be the plausible reason behind the weight loss in the disease control groups (Tables 1 and 2). While *Vasant Kusumakar Ras* and standard drug-treated groups showed an exponential feed intake pattern throughout the study (Table 2). A similar phenomenon was observed with the water intake (Table 2). There was a dose-dependent decrease in the fasting blood glucose levels with *Vasant Kusumakar Ras* treatment indicating its anti-diabetic

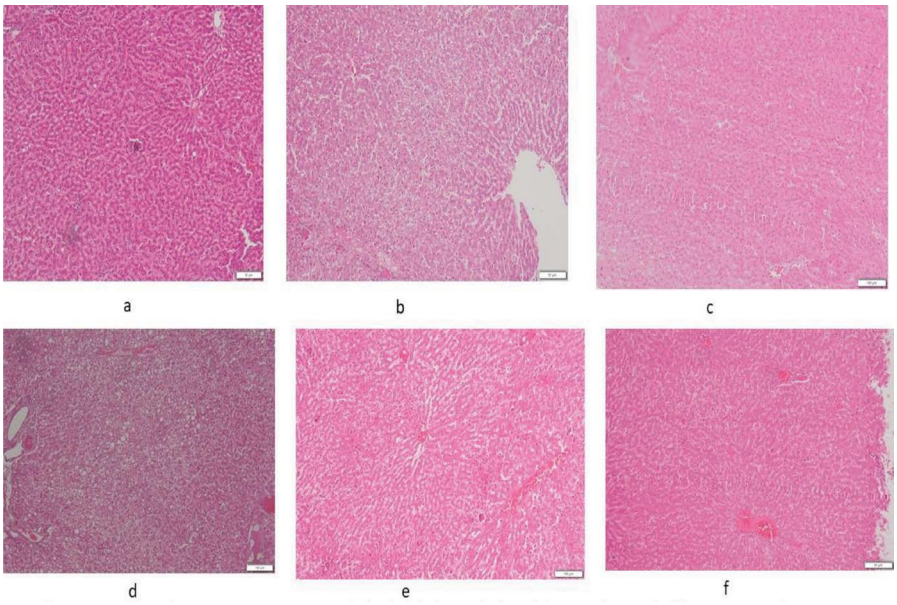

Figure 10. Effect of VK Ras treatment on pathological lesions of Liver **(a).** Normal control; **(b).** Streptozotocin (STZ + HFD) control; **(c).** High dose of VK Ras; **(d).** Average dose of VK Ras; **(e).** Low dose of VK Ras; **(f).** Glibenclamide.

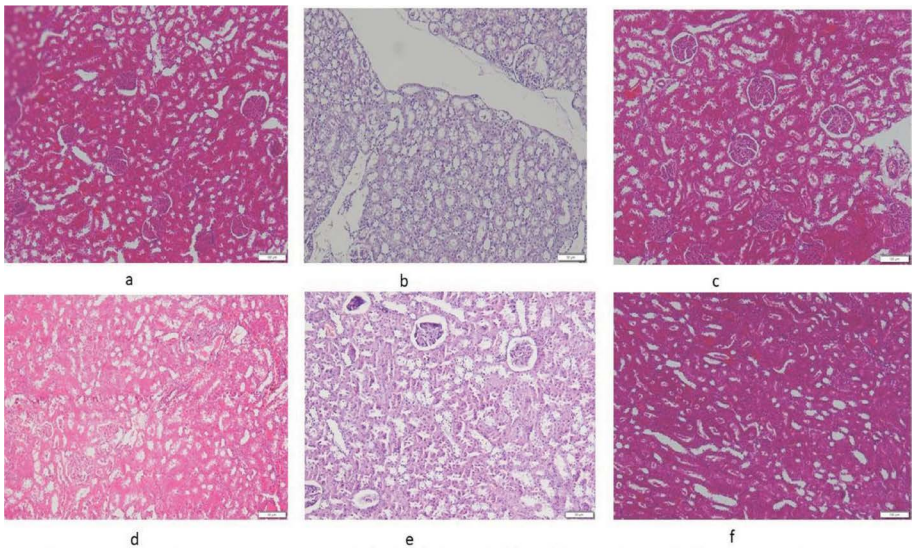

Figure 11. Effect of VK Ras treatment on pathological lesions of Kidney **(a).** Normal control; **(b).** Streptozotocin (STZ + HFD) control; **(c).** High dose of VK Ras; **(d).** Average dose of VK Ras; **(e).** Low dose of VK Ras; **(f).** Glibenclamide.

potential (Table 3). To this finding, plasma insulin levels in the disease-control animals were significantly lower and there was a dose-dependent improvement with treatments (Table 5). HOMA-IR and HbA1C findings were also in confirmation with previous results (Table 5). These findings indicate that *Vasant Kusumakar Ras* restored the insulin secretive activity of the pancreas. Streptozotocin induces pancreatic beta cell destruction by redox imbalance and subsequent oxidative stress[14]. Gold-containing ayurvedic formulations have potent anti-inflammatory activity[15]. Collectively, the contribution of Suvarna Bhamsa of *Vasant Kusumakar Ras* can be attributed to the cytoprotective effect on the pancreas. Histopathological analysis of the

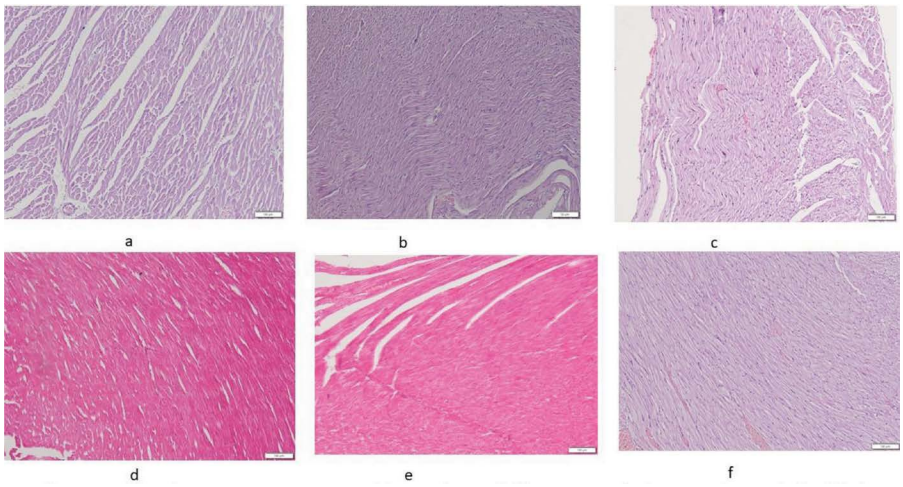

Figure 12. Effect of VK Ras treatment Heart **(a).** Normal control; **(b).** Streptozotocin (STZ + HFD) control; **(c).** High dose of VK Ras; **(d).** Average dose of VK Ras; **(e).** Low dose of VK Ras; **(f).** Glibenclamide.

pancreas of disease control animals showed mild to moderate reduction in size with degeneration of Islets of Langerhans indicating beta cell destruction (Figure 9). Treatment with *Vasant Kusumakar Ras* restored the histological architecture of the pancreas. Conclusively, it is to be noted that *Vasant Kusumakar Ras* improved the beta cell function and thereby restored the insulin levels (Figure 9).

The relationship between the liver and diabetes is indeed complex and bi-directional. Studies have shown an altered lipid metabolism and glycogen-associated disorders in non-alcoholic fatty liver subjects sharing similarities with metabolic derangement seen in type 2 diabetes. This makes liver function tests an essential part of the clinical evaluation of a diabetic subject. Liver function tests are a series of biochemical assays which measure different enzymes, proteins, and other substances made by the liver. Levels of Liver function markers such as AST, ALT and ALP are known to be distressed in diabetic subjects[16].

Correspondingly, High Fat Diet fed-streptozotocin-induced diabetic animals showed higher circulating levels of AST, ALT and ALP. Upon treatment with *Vasant Kusumakar Ras* produced a dose-dependent decrease in the liver markers (Table 4).

Glucose is stored in its polymeric form as glycogen in the liver and muscles in response to insulin. When blood glucose levels are lower glycogen breakdown into glucose and are released in the bloodstream in response to glucagon[17]. Correspondingly, diabetic rats showed an acute decrease in liver glycogen levels which was restored with *Vasant Kusumakar Ras* treatment in a dose-dependent manner (Table 6).

Persistent higher blood glucose levels result in Diabetic Nephropathy (DN) which is a syndrome characterized by progressive loss of kidney function. A recent study identified that 40% of type 2 diabetes mellitus subjects and 30% of type 1 diabetes subjects are prone to diabetic nephropathy and subsequent End Stage Renal Failure (ESRF)[18].

Creatinine is mainly found in skeletal muscle. The primary metabolite of creatinine is serum creatinine. Blood creatinine levels are directly correlated with skeletal muscle mass. Higher creatinine levels are observed during excess muscle breakdown[19]. The lower the mass of skeletal muscles decreases the targets for insulin binding and action. This may lead to insulin resistance development of type 2 diabetes[20]. However, after the onset of diabetic nephropathy, there will be an upshot in the serum creatinine levels[21]. It is to be noted that, histopathological changes correlating to circulating kidney injury markers can be observed from the 5th-week post streptozotocin injection in experimental animals[22]. Similarly, diabetic rats showed an acute increase in serum creatinine levels which was restored with *Vasant Kusumakar Ras* treatment in a dose-dependent manner (Table 6). As mentioned earlier, the creatinine levels are linked with the muscle mass of the animal, the mass of the soleus and digitorus extensor longus muscles was altered in diabetic rats and remained unchanged with the treatment (Table 7). The relative organ weight analysis of the heart, liver and kidney was

also performed to rule out any gross tissue changes as well as autophagy. It was observed that relative heart weight was unaltered in diabetic rats while the relative liver and kidney weights were increased indicating the onset of diabetic nephropathy and hepatomegaly. Treatment with *Vasant Kusumakar Ras* corrected the organ weights in a dose-dependent manner indicating the prevention of diabetic nephropathy and hepatomegaly (Table 7).

Additionally, the study also attempted to understand the effect of *Vasant Kusumakar Ras* on intestinal glucose uptake. According to the published studies, the rats with diabetes induced through HFD and low-dose STZ showed increased absorption of glucose in the small intestine[23]. Correspondingly the treatment with *Vasant Kusumakar Ras* opposed this phenomenon (Table 6).

6. Conclusions

Conclusively, the study highlighted a standardized high-fat diet- low dose streptozotocin rodent diabetic model and assessed the anti-diabetic potential of *Vasant Kusumakar Ras*. The findings of the study emphasized the significance of *Vasant Kusumakar Ras* in the prevention of diabetic complications such as Nephropathy and Hepatomegalopathy. Results of the study specify the prominence of *Vasant Kusumakar Ras* in decreasing intestinal glucose uptake and thereby regulating blood glucose levels. To summarize, the finding of the study concludes that *Vasant Kusumakar Ras* is an anti-diabetic agent with an organo-protective action.

7. Acknowledgements

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8. References

1. Oschatz E, Müllner M, Herkner H, Laggner AN. Multiple organ failure and prognosis in adult patients with diabetic ketoacidosis. Wien Klin Wochenschr. 1999; 111(15):590-5. PMID: 10483673.
2. Leon BM, Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes. 2015; 6(13):1246-58. https://doi.org/10.4239/wjd.v6.i13.1246 PMid:26468341 PMCid:PMC4600176
3. Chaudhary A, Singh N. Contribution of World Health Organization in the global acceptance of Ayurveda. J Ayurveda Integr Med. 2011; 2(4):179-186. https://doi.org/10.4103/0975-9476.90769 PMid:22253507 PMCid: PMC3255448
4. McWhorter LS. Biological complementary therapies: A Focus on botanical products in diabetes. Diabetes Spectrum. 2001; 14:199-208. https://doi.org/10.2337/diaspect.14.4.199
5. Saxena A, Vikram NK. Role of selected Indian plants in management of type 2 diabetes: a review. Journal of Alternative and Complementary Medicine. 2004; 10:369-78. https://doi.org/10.1089/107555304323062365 PMid:15165418
6. Sarkar PK, Das S, Prajapati PK. Ancient concept of metal pharmacology based on Ayurvedic literature. Anc Sci Life. 2010; 29(4):1-6
7. Tamoli SM, Kohli KR, Kaikini AA, Muke SA, Shaikh AA, Sathaye S. *Vasant Kusmakar Ras*, an ayurvedic herbo-mineral formulation prevents the development of diabetic retinopathy in rats. J Ayurveda Integr Med. 2020; 11(3):270-276. https://doi.org/10.1016/j.jaim.2020.02.002 PMid:32312587 PMCid:PMC7527844
8. Winzell MS, Ahren B. The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes. 2004; 53(Suppl. 3):S215-S219. https://doi.org/10.2337/diabetes.53.suppl_3.S215 PMid:15561913
9. Kanno A, Asahara SI, Masuda K, Matsuda T, Kimura-Koyanagi M, Seino S, Ogawa W, Kido Y. Compensatory hyperinsulinemia in high-fat diet-induced obese mice is associated with enhanced insulin translation in islets. Biochem Biophys Res Commun. 2015; 458(3):681-686. https://doi.org/10.1016/j.bbrc.2015.02.024 PMid:25686499
10. Brøndum E, Nilsson H, Aalkjaer C. Functional abnormalities in isolated arteries from Goto-Kakizaki and streptozotocin-treated diabetic rat models. HormMetab Res. 2005; 37 (Suppl 1):56-60 https://doi.org/10.1055/s-2005-861370 PMid:15918112
11. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacological Research. 2005; 52(4):313-320. https://doi.org/10.1016/j.phrs.2005.05.004 PMid:15979893
12. Doluisio JT, Billups NF, Dittert LW, Sugita ET, Swintosky JV. Drug absorption. I. An in-situ rat gut technique yielding realistic absorption rates. J Pharm Sci. 1969; 58(10):1196-200. https://doi.org/10.1002/jps.2600581006 PMid:5394662
13. Van der vies J. Two methods for the determination of glycogen in liver. Biochem J. 1954; 57(3):410-6. https://

doi.org/10.1042/bj0570410 PMid:13181850 PMCid: PMC1269772
14. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008; 51(2):216-26. https://doi.org/10.1007/s00125-007-0886-7 PMid:18087688
15. Strong JS, Bartholomew BA, Smyth CJ. Immuno-responsiveness of patients with rheumatoid arthritis receiving cyclophosphamide or gold salts. Ann Rheum Dis. 1973; 32(3):233-237. https://doi.org/10.1136/ard.32.3.233 PMid:4715541 PMCid:PMC1006085
16. Mandal A, Bhattarai B, Kafle P, Khalid M, Jonnadula SK, Lamicchane J, Kanth R, Gayam V. Elevated liver enzymes in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease. Cureus. 2018 2018; 10(11). https://doi.org/10.7759/cureus.3626
17. Ferrannini E, Lanfranchi A, Rohner-Jeanrenaud F, Manfredini G, Van de Werve G. Influence of long-term diabetes on liver glycogen metabolism in the rat. Metabolism. 1990; 39(10):1082-8. https://doi.org/10.1016/0026-0495(90)90170-H PMid:2145494
18. Hussain S, Jamali MC, Habib A, Hussain MS, Akhtar M, Najmi AK. Diabetic kidney disease: An overview of prevalence, risk factors, and biomarkers. Clinical Epidemiology and Global Health. 2021; 9:2-6. https://doi.org/10.1016/j.cegh.2020.05.016
19. Harita N, Hayashi T, Sato KK, Nakamura Y, Yoneda T, Endo G, Kambe H. Lower serum creatinine is a new risk factor of type 2 diabetes: The Kansai healthcare study. Diabetes Care. 2009; 32:424-426. https://doi.org/10.2337/dc08-1265 PMid:19074997 PMCid:PMC2646021
20. DeFronzo RA, Gunnarsson R, Björkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest. 1985; 76:149-155. https://doi.org/10.1172/JCI111938 PMid:3894418 PMCid:PMC423730
21. Onuigbo MA, Agbasi N. Diabetic nephropathy and CKD-analysis of individual patient serum creatinine trajectories: A forgotten diagnostic methodology for diabetic CKD prognostication and prediction. J Clin Med. 2015; 4(7):1348-68. https://doi.org/10.3390/jcm4071348 PMid: 26239680 PMCid:PMC4519794
22. Ni Z, Guo L, Liu F, Olatunji OJ, Yin M. Allium tuberosum alleviates diabetic nephropathy by supressing hyperglycemia-induced oxidative stress and inflammation in high fat diet/streptozotocin treated rats. Biomed Pharmacother. 2019; 112. https://doi.org/10.1016/j.biopha.2019.108678 PMid:30784905
23. Gromova LV, Polozov AS, Kornyushin OV, Grefner NM, Dmitrieva YV, Alekseeva AS, Gruzdkov AA. Glucose absorption in the rat small intestine under experimental Type 2 Diabetes Mellitus. J Evol Biochem Physiol. 2019; 55:155-157. https://doi.org/10.1134/S0022093019020091